PHOSPHATE MODULE: LAB NOTEBOOK WEEKLY SUMMARY

Week 1 (1/6/2015-5/6/2015)

1. Summer training, mainly for recalling the basic techniques

2. Discussion for the plan of the main project

Week 2 (8/6/2015-12/6/2015)

1. Transformation of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840

2. Inoculation of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840

3. Plasmid extraction of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840

4. Phosphorylation and annealing of *phoAp*

5. Digestion of pSB1C3-BBa_J04450

6. Ligation of *phoAp* with pSB1C3 and pSB1C3-BBa_I0500 with pSB1C3-BBa_B0030

Week 3 (15/6/2015-19/6/2015)

1. Transformation of pSB1A2-BBa_B0030, pSB1A2-BBa_E0840, pSB1AK3-BBa_B0015

2. Phosphorylation and annealing of *phoAp*

3. Digestion of pSB1C3-BBa_J04450

Week 4 (22/6/2015-26/6/2015)

1. Colony PCR for checking the identity of pSB1C3-*phoAp* candidate 1 and 3 by VF_2 and VR primers

2. Restriction check of pSB1C3-*phoAp* candidate 1 and 3 using PvuII

Week 5 (29/6/2015-3/7/2015)

1. Digestion of pSB1C3-BBa_B0030.

2. Digestion of pSB1C3-BBa_J04450.

3. Overnight ligation of *phoAp* with pSB1C3 at $16^{o}C$.

4. Transformation of pSB1C3-*phoAp*.

5. Colony PCR for checking the identity pSB1C3-*phoAp* candidate colonies. Identified candidate 1-17 of pSB1C3-*phoAp*.

6. Digestion of pSB1C3-BBa_B0030

7. New plan for *phoAp* , instead of having the construct of pSB1C3-*phoAp* for sequencing, rather, ligate *phoAp* with pSB1A2-BBa_E0840 for sequencing.

8. Digestion of pSB1A2-BBa_E0840

9. Overnight ligation of pSB1A2-BBa_E0840 with *phoAp* at 16^{o}C.

Week 6 (6/7/2015-10/7/2015)

1. Transformation of pSB1A2-*phoAp*-BBa_E0840.

2. Identified pSB1A2-*phoAp*-BBa_E0840 candidates by restriction check using PvuII.

3. Miniprep of pSB1A2-*phoAp*-BBa_E0840 candidate 1-5.

4. Ligation of pSB1A2-BBa_E0840 with *phoAp*.

5. Send pSB1A2-*phoAp*-BBa_E0840 candidate 5 for sequencing.

6. Inoculation and streak pSB1C3-BBa_B0030, pSB1AK3-BBa_B0015

Week 7 (13/7/2015-17/7/2015)

1. Send pSB1A2-P_{phoA}-BBa_E0840 for sequencing.

2. Confirmed pSB1A2-*phoAp*-BBa_E0840 from the sequencing result.

3. From sequencing result of pSB1C3-BBa_B0030, it showed extra nucleotides before and after the BBa_B0030 sequence, which is inconsistent with the sequence from the iGEM Parts Registry.

4. Transformation of pSB1C3-BBa_B0032.

5. Miniprep of pSB1C3-BBa_B0032 and pSB1C3-BBa_B0030.

6. Digestion of pSB1C3-BBa_B0032.

7. Glycerol stock for pSB1AK3-BBa_B0015.

8. Functional assay for *phoAp*.

9. Characterization of *phoAp.*

10. Prepared modified M9 minimal medium for P_{phoA} characterization with phosphate being replaced.

Week 8 (20/7/2015-24/7/2015)

1. Innoculate pSB1A2-*phoAp*-BBa_E0840 in M9 minimal medium with 0µM, 10µM, 500µM

and 1000μM of PO_4 (Using Tris hydrochloride to replace the original PO_4 in M9)

2. Check OD and GFP expression

3. Digestion and gel purification of pSB1A2-BBa_E0840 (for ligating with pSB1A2-BBa_J23101 to act as a positive control)

Week 9 (27/7/2015-31/7/2015)

1. Characterization of pSB1A2-*phoAp*-BBa_E0840 in M9 minimal medium with 0μM, 30μM, 50μM, 100μM, 150μM, 200μM, 250μM PO_4^{3-}.

2. Digestion of pSB1A2-BBa_J23101-J61002 (constitutive promoter-RFP reporter)

3. Ligation of BBa_J23101 (constitutive promoter) and BBa_E0840(GFP reporter)

4. Streak plates for pSB1A2-*phoAp*-BBa_E0840 candidate 5, pSB1A2-E0840 and pSB1A2-BBa_J23101-E0840

Week 10 (3/8/2015-7/8/2015)

1. Characterization of pSB1A2-BBa_P_{phoA}-E0840.

Week 11 (10/8/2015- 14/8/2015)

1. Passed to Rice University.

Week 12 (17/8/2015- 23/8/2015)

1. Continued the work of phosphate sensor from Rice University.

6. Characterization on *phoAp* promoter.

7. Characterization on *phoBRp* promoter.

8. Characterization on *AMNp* promoter.

Week 13 (24/8/2015-30/8/2015)

1. Characterization on *phoAp* promoter.

2. Characterization on *phoBRp* promoter.

3. Characterization on *AMNp* promoter.

Week 14 (31/8/2015-6/9/2015)

1. Characterization on *phoAp* promoter.

2. Characterization on *phoBRp* promoter.

3. Characterization on *AMNp* promoter.